Louisiana Office of Public Health Laboratories	
Test Name	Trioplex Real Time rt-PCR
PHL Location	Office of Public Health Laboratory Baton Rouge
CPT Code	87798 x 3
Synonyms	Zika, Chik, Dengue
Brief Description of Test	**Prior authorization required. Contact Infectious Disease Epidemiology at 800-256-2748.** The Trioplex Real-Time RT-PCR assay is used on the ABI 7500 Fast Dx Real-Time PCR Instrument. This assay tests for the presence of Zika, Chikungunya and Dengue virus.
Possible Results	**Trioplex rRT-PCR Interpretation and Reporting Instructions for Serum and CSF Specimens**

ZIKV	DENV	CHIKV	RP	Interpretation	Reporting	Actions
-	-	-	+	Negative	No Zika, dengue, or chikungunya RNA detected by rRT-PCR	Report results to CDC. No further testing required. Note: If date of onset of symptoms is in doubt or if patient is asymptomatic, serological testing may be recommended. Refer to CDC algorithm.*
-	-	-	-	Inconclusive	Specimen inconclusive for the presence of Zika, dengue, and chikungunya RNA by rRT-PCR. An inconclusive result may occur in the case of an inadequate specimen.	Repeat extraction and rRT-PCR. If unable to resolve inconclusive result for a serum specimen, request collection of additional serum from the patient. Report inconclusive results to CDC.
-	+	-	+/-	Positive for DENV, but negative for ZIKV and CHIKV.	Dengue RNA detected by rRT-PCR. No Zika or chikungunya RNA detected.	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*
-	-	+	+/-	Positive for CHIKV, but negative for ZIKV and DENV.	Chikungunya RNA detected by rRT-PCR. No dengue or Zika RNA detected.	
+	-	-	+/-	Positive for ZIKV, but negative for DENV and CHIKV.	Zika RNA detected by rRT-PCR. No dengue or chikungunya RNA detected.	
-	+	+	+/-	Positive for DENV and CHIKV, but negative for ZIKV.	Dengue and chikungunya RNA detected by rRT-PCR. No Zika RNA detected.	
+	+	-	+/-	Positive for ZIKV and DENV, but negative for CHIKV	Zika and dengue RNA detected by rRT-PCR. No chikungunya RNA detected.	
+	-	+	+/-	Positive for ZIKV and CHIKV, but negative for DENV	Zika and chikungunya RNA detected by rRT-PCR. No dengue RNA detected.	
+	+	+	+/-	Positive for ZIKV, DENV, and CHIKV	Zika, dengue, and chikungunya RNA detected by rRT-PCR.	

Trioplex rRT-PCR Interpretation and Reporting Instructions for Urine and Amniotic Fluid Specimens

ZIKV	RP	Interpretation	Reporting	Actions
-	+	Negative	No Zika RNA detected by rRT-PCR.	Report results to CDC. Refer to CDC algorithm.*
-	-	Inconclusive	Specimen inconclusive for the presence of Zika RNA by rRT-PCR. An inconclusive result may occur in the case of an inadequate specimen.	Repeat extraction and rRT-PCR. If repeat testing does not resolve inconclusive result, do not test further. Report results to CDC.
+	+/-	Positive	Zika RNA detected by rRT-PCR	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*

Reference Range	Negative
Specimen Type	Serum is the preferred diagnostic specimen. CSF, urine and amniotic fluid may only be tested alongside a patient-matched serum specimen.
Specimen Container(s):	Red top tubes, Marble top tubes, polypropylene vials
Minimum volume accepted:	300 µL
Collection Instructions	Specimens should only be collected by personnel that have been properly trained. Care should be taken during specimen collection and handling to avoid generation of aerosols. Blood should be collected in a plastic tube, such as a vacutainer, which does not contain an anticoagulant. The collection tube may or may not contain a serum separator. If collected in a tube without serum separator, serum must be aliquoted into screw cap tubes before shipment to laboratory. Depending on the type of collection tube, the amount of time it will take for the blood to clot could take up to 60 minutes. Separation of serum from cells should take place within 2 hours of collection to prevent erroneous test results according to NCCLS guidelines. Follow the package insert for the collection tube you use. Specimens other than serum should be collected in polypropylene vials. Label specimen with Patient Name and a 2nd unique identifier such as a chart number or medical record number. DOB is not considered unique. Complete a Lab Form 96 to accompany the sample(s). Each specimen must have a separate lab submission form. Lab submission forms must be thoroughly completed with patient's first and last name, 2nd patient identifier, gender, date of birth, date of collection, time of collection, onset date, test requested (TRIOPLEX), and submitter's name, address, and contact number. Two unique identifiers **MUST** be recorded on the tube **AND** the Lab 96 form. A missing identifier on the tube will be an automatic rejection. If the identifiers are missing from the Lab 96 form, the submitter must be contacted and a new form with this information must be faxed back to the lab before testing will take place.

	Transport specimen to laboratory as soon as possible after collection. Keep submission forms insulated from specimens.
Storage and Transport Instructions	Once there is a clinical diagnosis of suspected Zika, take a venous, whole blood sample. Follow serum specimen collection devices manufacturer instructions for proper collection, separation and storage methods. We recommend that separated serum samples are frozen at -20°C and sent or shipped in dry ice to the testing laboratories. If dry ice is not available, we recommend that separated serum is maintained on ice or in a refrigerator for no longer than 2 hours before it is either frozen at -20° C or tested. • Transport/ship human serum samples in dry ice. Document the date and time sample was frozen. For this assay, if dry ice is not available, samples will be accepted on cold-packs.
Causes for Rejection	Unspun samples, tubes that contain less than 90% of the total drawing capacity (QNS), incorrect specimen type, or expired collection tubes must be rejected. Improper storage and improper transport temperature requirements are also reasons for rejection.
Limitations of the Procedure	This assay was approved via an Emergency Use Authorization. This assay is very dependent on the timing of specimen collection. Specimens collected within 7 days of illness onset will yield the best results.
Interfering Substances	N/A
References	Package Insert: CDC Trioplex Real Time RT-PCR Assay Trioplex FDA Letter of Authorization – March 17, 2016
Additional Information	Fact Sheets for Providers can be located at: http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491588.pdf Fact Sheets for Patients can be located at: http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491590.pdf Fact Sheets for Pregnant Women can be located at: http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491591.pdf
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